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Comparison of Chinese Field Strains of Avian Leukosis Subgroup J Viruses with Prototype Strain HPRS-103 and United States Strains

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SUMMARY. Eight Chinese field strains of subgroup J avian leukosis viruses (ALV-J) were isolated from broilers or parent stocks during January 1999 to April 2001. One strain, SD9902, was an acute transforming virus, able to induce typical myelocytomatosis in 22–38 days after inoculation of 1-day-old meat-type chicks. The envelope protein and 3′-untranslated region (UTR) of the eight field strains were compared with the U.K. prototype HPRS-103 and several U.S. field strains isolated in 1993–97. All Chinese strains shared an almost identical deletion with the U.S. strain 4817 in the E element region of 3′-UTR when compared with the prototype HPRS-103, indicating that they have a very close phylogenic relationship. Every year, China has to import grandparent stocks of meat-type chickens, mainly from the United States. Chinese isolates should represent a part in the phylogenic tree of U.S. ALV-J evolution. Envelope protein gp85 amino acid sequence analysis demonstrated that, interestingly, all recent Chinese isolates were more closely related to HPRS-103 and the earliest U.S. isolates but not to the late U.S. isolates. The result implies that envelope gp85 may not have diverged from prototype and older strains. It is also possible that some recently imported birds could have been infected by the older viruses that were introduced in the late 1990s.

RESUMEN. Comparación de los aislamientos de campo del virus de la leucosis aviar del subgrupo J provenientes de China, con la cepa prototipo HPRS-103 y cepas provenientes de los Estados Unidos.

Ocho cepas de campo del virus de la leucosis aviar del subgrupo J (ALV-J) de China fueron aisladas a partir de parvadas de pollos de engorde y reproductoras entre Enero de 1999 y Abril del 2001. Una de las cepas, la SD9902, es un virus oncogénico capaz de producir mielocitomatosis típica en forma aguda de 22 a 38 días después de la inoculación en aves de engorde al día de edad. Se comparó la proteína de la envoltura y la región no traducida de la parte 3′ (3′-UTR por sus siglas en Inglés) de ocho cepas de campo con las de la cepa prototipo Inglesa HPRS-103 y varias cepas de campo de los Estados Unidos aisladas en el periodo comprendido entre los años 1993–97. Todas las cepas aisladas en China presentaron una secuencia de nucleótidos del elemento E de la 3′-UTR casi idéntica a la cepa estadounidense 4817, las cuales presentan una secuencia truncada en comparación con la cepa HPRS-103, indicando que existe una correlación filogenética cercana entre las mismas. Cada año China tiene que importar parvadas de abuelos para la producción de reproductoras de engorde, principalmente desde los Estados Unidos. Los aislamientos de campo de China deberían representar una parte del árbol filogenético en evolución de los aislados de ALV-J de los Estados Unidos. El análisis de la secuencia de aminoácidos de la proteína de la envoltura gp85 demostró que todos los aislados recientes provenientes de China presentaron una relación más cercana con las cepas HPRS-103 y aislamientos tempranos del virus provenientes de los Estados Unidos que con aislamientos recientes del virus proveniente de este país. Estos resultados sugieren que la secuencia de la proteína de la envoltura gp85 pudo no haber divergido de la cepa prototipo y

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cepas más viejas del virus. También es posible que las aves importadas recientemente pudieron haber sido infectadas por cepas virales viejas, introducidas a China al final de la década del 1990.

Key words: ALV-J, DNA sequence, proviral genome, field isolates, phylogeny

Abbreviations: aa = amino acids; ALV = avian leukosis virus; ALV-J = subgroup J avian leukosis virus; CEF = chicken embryo fibroblast; IFA = indirect fluorescence antibody; LTR = long terminal repeat; NS/S ratio = nonsynonymous nucleotide changes *vs.* synonymous nucleotide changes; PCR = polymerase chain reaction; rTM = repeated trans-membrane; UTR = untranslated region

Avian leukosis virus (ALV) subgroup J (ALV-J) was first isolated in 1988 from meat-type chickens in Great Britain (9). It was differentiated from classic ALV subgroups A, B, C, D, and E by neutralization and interference assays (9,11) and then by genomic sequences (1,2). HPRS-103, the prototype strain of ALV-J, induced mainly myelocytomatosis and nephromas in meat-type chickens. During the last 10 yr, ALV-J has been recognized and reported in most parts of the world. In the field, it causes both late-onset myeloid tumors and acute tumors even in 4-to-5-wk-old birds (8,10). ALV-J has mutated much faster than other classic subgroups, and high variability in envelope glycoproteins gp85 and gp37 was reported (4,16,18). By comparison with genomic sequences of U.K. and U.S. isolates, researchers concluded that all tested ALV-J isolates probably arose from a common ancestor (3,16) and envelope glycoproteins of U.S. strains were slowly drifting away from their progenitor (16). To understand whether further emerging ALV-J would continue the trend, there is a need to analyze more samples.

China has the second largest broiler industry in the world, and all broiler breeders were imported from different parts of the world during the last two decades. Nevertheless, ALV-J was not officially recognized in China until 1999 (6). In the last 3 yr, we obtained 10 ALV-J isolates from broiler breeders and commercial broilers in Shandong, Jiangsu, Henan, and Ninxia provinces. Actually, the typical myelocytomatosis lesions in livers had been seen by poultry farm veterinarians in the early 1990s. In the last 10 yr, because most broiler breeders are imported from the United States, ALV-J isolates obtained recently in China should belong to the same phylogenic family of current U.S. isolates. In this study, we compared eight Chinese and five U.S. isolates obtained in different years with the prototype HPRS-103 for their envelope glycoproteins and 3′-untranslated region (UTR) of the genomic proviral DNA. This comparison should help us to further understand the continued evolution trend of the virus.

MATERIALS AND METHODS

Background of eight Chinese ALV-J isolates. As shown in Table 1, all eight Chinese strains were isolated during January 1999 to April 2001 from four provinces in China; some of them were reported previously (6,7). Except for isolate YZ9901, which was isolated from commercial broilers at slaughter, all were isolated from dead birds with lesions suspected to be due to ALV-J infection. The birds belonged to different broiler strains from three different companies that we coded A, B, and C. The primary parent stocks of company A were imported from the United States before 1995 and have been closely raised since then. But the grandparent stocks of company B and company C have been imported into China every year. Among eight strains, isolate SD9901 was confirmed to be an acute transforming virus in a bird experiment (5).

Virus isolation and identification. Livers and spleens were collected from suspected dead broiler breeder chickens, or from commercial broilers at slaughter. Tissue suspensions in phosphate-buffered saline were filtered through 0.45-μm filters and inoculated onto chicken embryo fibroblast (CEF) (from line 0 chickens or SPAFAS specific-pathogen-free chickens) cultures. The inoculated CEF cultures were trypsinized and harvested after they were kept in a CO_2 incubator at 37 C for 10–14 days with medium changed three times. The DNA samples were extracted separately from each inoculated CEF pellet and used as template for polymerase chain reaction (PCR). The ALV-J specific primers used were the forward primer "6" (5′-CTT GCT GCC ATC GAG AGG TTA CT-3′) and reverse primer "2" (5′-AGT TGT CAG GGA ATC GAC-3′). The PCR procedures were as previously reported (16). Demonstration of an approximate 2.2-kb band in gel electrophoresis of PCR products was considered primary evidence for ALV-J infection in the CEF cultures. At the same time, indirect fluorescence antibody (IFA) tests with an ALV-J specific monoclonal antibody JE9 (12) were conducted on the coverslips to confirm the ALV-J infections.

DNA sequencing and analysis. PCR products of about 2.2 kb were cloned into TA vector pCR2.1 (Invitrogen) and sent to some commercial service companies in cities of Shanghai and Dalian in China for sequencing by automatic DNA sequencers. Strains YZ9901, SD9901, and SD9902 were sequenced three

Table 1. The background of eight Chinese strains of ALV-J.

Strain	Year isolated[A]	Samples from chickens: Company[B]	Type[C]	Age (weeks)	Tumor lesions	Myelocytoma cells in sections[D]	IFA with Mab JE9
YZ9901	1999.1	B?	C	7	From slaughterhouse, no typical lesion	Not done	+
SD9901	1999.8	A	PS	45	Many small tumor spots in liver and spleen	Yes	+
SD9902	1999.8	A	PS	45	Many small tumor spots in liver and spleen	Yes	+
SD0001	2000.5	?	PS	30	Many tumor nodules in liver and spleen, on ribs and sternum	Yes	+
SD0002	2000.8	A?	C	4–5	Many small tumor spots in liver and spleen	Yes	+
HN0001	2000.11	C	PS	26	Many small tumor spots in liver and spleen	Yes	+
SD0101	2001.2	B	PS	55	Many small tumor spots in liver and spleen	Yes	+
NX0101	2001.4	A	PS	20	Many tumor nodules in liver and spleen, on ribs and sternum	Yes	+

[A]The number after the decimal point indicates the month of isolation.
[B]Companies A, B, and C were according to the clear background history; company A? and B? were assumed origin, according to both gp85 and 3′-LTR deletion analysis.
[C]C = commercial broilers; PS = parent stocks.
[D]See reference 12.

times by different companies to confirm each other's sequence data. The other five isolates were sequenced only once. The five U.S. strains were Hc1, 0661, 4817, 6683, and 6827, representing isolates obtained in 1993–97 (8). Their envelope proteins, gp85 and gp37, have been reported before (16). The 3′-UTRs of these strains were sequenced at the Avian Disease and Oncology Laboratory. The envelope protein and 3′-UTR sequences of all the above strains were compared with the published sequence of the U.K. prototype strain HPRS-103 (1,2). Both envelope protein and 3′-UTR DNA sequences of 14 strains were aligned and compared by the Clustal V method in MegAlign of DNASTAR program (Madison, WI).

Influence of selection pressures on gp85 mutations. To determine the selection pressures on ALV-J viral quasispecies, the nucleotide substitution patterns of gp85 genes of eight Chinese strains and five U.S. strains compared with prototype HPRS-103 were analyzed by calculation of the NS/S ratio (nonsynonymous nucleotide changes *vs.* synonymous nucleotide changes) as described in detail by Venugopal and coworkers (18).

RESULTS

Comparison of the envelope glycoprotein gp85 in Chinese and U.S. strains with the U.K. prototype HPRS-103. Fig. 1 and Table 2 demonstrate the evolutional relationships of gp85 amino acid sequences of eight Chinese strains isolated in 1999–2001, five U.S. field strains isolated during 1989–97, and the prototype U.K. HPRS-103 isolated in 1989. The gp85 amino acid sequence identities were in the range of 88.2%–98.4% when they were compared with each other. The eight Chinese strains from three different companies, A, B, and C (Table 1), were located in three separated groups in the phylogenetic tree (Fig. 1). The gp85 identities among four strains, SD9901, SD9902, SD0002, and NX0101, from company A were in the range of 91.9%–98.4%, and the highest identity of 98.4% was seen between strains SD9902 and SD0002.

Table 2 also shows the gp85 relationship of eight Chinese strains compared with the U.K. prototype, HPRS-103 (88.9%–94.2%), the earliest known U.S. strain, Hc1 (88.9%–91.2%), and the late U.S. strain 6683 (87.3%–91.9%). The U.S. strain 6827, isolated in 1997, was a unique isolate, having very low gp85 identities with the eight Chinese strains (82.1%–86.0%) and the other U.S. or U.K. strains (82.6%–85.7%).

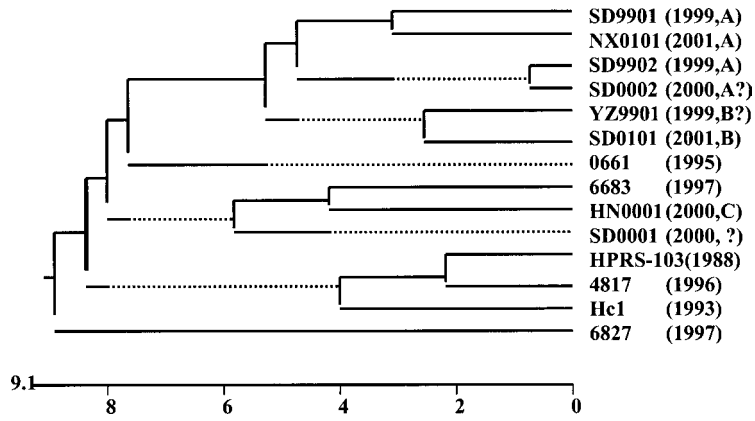

Fig. 1. Phylogenetic relationship of gp85 amino acid sequences of eight Chinese strains and five U.S. strains compared with the prototype strain HPRS-103 of ALV-J. In parentheses after the strain name, the year isolated and company (for Chinese strains if known) are indicated (analyzed by Clustal method of DNAStar).

Comparison of the envelope glycoprotein gp37 of Chinese and U.S. strains with the U.K. prototype HPRS-103. The amino acid identities of the gp37 amino acid sequence among eight Chinese strains were in the range of 93.9%–99.0%. When compared with the U.K. HPRS-103 and five U.S. strains, the gp37 identities were in the range of 90.4%–95.9% and 88.0%–97.0% (Table 3). Among four strains, SD9901, SD9902, SD0002, and NX0101, from company A in China, the gp37 identity was as high as 98.5%. The locations of eight Chinese strains in the phylogenetic tree of gp37 amino acid sequence were similar to gp85 but not exactly corresponding to the origin of the chicken breeds. The notable exception in this comparison is that the highest gp37 identity of 99.0% was between strains SD9902 (from company A) and SD0101 (from company B).

Comparison of deletions in 3′ end of the genomic proviral DNA of Chinese and U.S. strains with the U.K. prototype HPRS-103. When compared with the prototype U.K. strain HPRS-103, all tested Chinese and U.S. strains have one or two deletions at the 3′ end of the genomic

Table 2. Comparison of eight Chinese strains and five U.S. strains with ALV-J prototype strain HPRS-103 for their gp85 amino acid sequence identity.[A]

% Identity														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	Strain
	92.5	87.8	95.5	89.3	84.4	89.9	94.2	93.8	91.6	92.5	89.2	88.9	90.3	1 HPRS-103
		88.2	90.2	87.6	85.7	90.9	91.1	89.9	89.6	89.9	88.9	89.5	89.9	2 Hc1
			88.2	88.8	82.6	88.5	89.1	88.5	88.2	88.5	88.8	89.5	86.2	3 0661
				90.2	84.7	88.6	92.9	93.2	92.9	93.2	87.9	87.9	89.0	4 4817
					84.0	87.3	90.9	91.5	91.9	90.9	91.2	89.2	88.3	5 6683
						82.8	84.1	84.1	86.0	84.4	85.9	84.6	82.1	6 6827
							92.2	91.9	90.3	91.6	88.6	92.8	89.9	7 YZ9901
								95.8	92.9	95.5	89.9	91.2	93.2	8 SD9901
									93.8	98.4	90.5	90.8	92.2	9 SD9902
										94.2	91.5	90.5	89.9	10 SD0001
											90.2	90.5	91.9	11 SD0002
												89.2	87.6	12 HN0001
													88.2	13 SD0101
														14 NX0101

[A]The ALV-J strains were arranged from 1 to 14 according to year of isolation. Strains 2–6 are U.S. strains isolated during 1993–97; strains 7–14 are Chinese strains isolated during 1999–2001.

Table 3. Comparison of eight Chinese strains and five U.S. strains with ALV-J prototype strain HPRS-103 for their gp37 amino acid sequence identity.[A]

% Identity														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	Strain
	95.9	92.9	90.4	91.4	92.4	92.4	93.4	94.9	92.9	93.4	92.4	94.9	93.4	1 HPRS-103
		95.5	92.4	94.9	96.0	95.5	96.5	98.0	95.4	96.4	95.9	97.0	96.4	2 Hc1
			89.9	93.9	94.4	93.9	95.5	95.4	94.9	95.9	94.9	94.4	94.9	3 0661
				91.4	91.9	88.0	89.5	92.4	91.9	90.9	92.9	91.4	90.4	4 4817
					96.5	93.9	95.5	95.9	94.4	95.9	94.4	94.9	94.4	5 6683
						94.4	96.0	97.0	95.9	95.9	95.9	95.9	94.4	6 6827
							98.5	97.0	93.9	97.0	93.9	97.0	98.0	7 YZ9901
								98.5	95.4	98.5	95.4	98.5	98.5	8 SD9901
									95.4	98.5	95.4	99.0	97.0	9 SD9902
										95.9	99.0	94.4	94.9	10 SD0001
											95.9	97.5	97.5	11 SD0002
												94.4	94.9	12 HN0001
													97.0	13 SD0101
														14 NX0101

[A]Strains 2–6 are U.S. strains isolated during 1993–97; strains 7–14 are Chinese strains isolated during 1999–2001. They are arranged from 1 to 14 according to year of isolation years.

proviral DNA (Fig. 2). Although the deleted fragments are not identical to each other, these deletions overlap between strains. Surprisingly, all eight Chinese strains have an almost identical deletion of 127 bp in the E element similar to U.S. strain 4817 isolated in 1996. However, all the Chinese strains have a unique deletion in the repeated transmembrane (rTM) region that differs from strain 4817 and the other U.S. strains. Fig. 3 shows both the nucleotide sequences flanking the deletion and the deleted sequences in the E element of U.S. strain 4817 and all eight Chinese strains compared with the prototype HPRS-103 and other U.S. strains. The deletions in the E elements are nearly identical, with only one or two base differences between the U.S. strain 4817 and all eight Chinese strains. In Fig. 4, sequence homologies among prototype HPRS-103, U.S. strain 4817, and Chinese strain SD9902 were compared at regions between the *env* and 3′-long terminal repeat (LTR). Strains SD9902 and 4817 are very similar at the right side of this region (98.9% homology), but SD9902 is closer to prototype HPRS-103 at the left side of this region.

Among the eight Chinese strains, three or four deletion patterns in the 3′ end of the genomic proviral DNA were identified (Fig. 2), all sharing a common deletion in the E element. Strain SD0002 (its flock origin was unknown) was located in the same group of the gp85 phylogenetic tree (Fig. 1) as strains SD9901, SD9902, and NX0101 from company A, and all these four strains had the same deletion pattern. There were three separate deletions of 2–3 bp each in the left side of the rTM region (sequence data not shown). Strain SD0101 (from company B) shared another deletion of 9 bp in the middle of the rTM region with strain YZ9901 (flock origin unknown). Both strains also mapped in the same group of the gp85 phylogenetic tree. The Chinese strains HN0001 and SD0001 contained a large deletion to the right side of the transmembrane region and in most of the rTM region. The strain HN0001 was from company C and had one additional deletion of 10 bp in the U3 region (Fig. 2). The map location of the gp85 in the phylogenetic tree and the deletion patterns around the E element were consistent with their origin. However, the 3′-LTRs of the genomic proviral DNA were much more conserved than their gp85 sequences. For example, the strains SD9901, SD9902, SD0002, and NX0101 shared a similar deletion pattern in the 3′-LTR, but their gp85 sequences differed from each other with an amino acid identity of 91.9%–98.4, a large difference of 8.1% between SD0002 and NX0101.

The selection pressure on gp85 of eight Chinese strains and five U.S. strains of ALV-J isolated in 1993–2001. The NS/S ratios for gp85 gene nucleotide changes in eight Chinese strains and five U.S. strains were compared with HPRS-103 and are listed in Table 4. The NS/S ratio of total gp85 gene substitutions was only 1.05 (112/107), indicating no evidence for selective pressure.

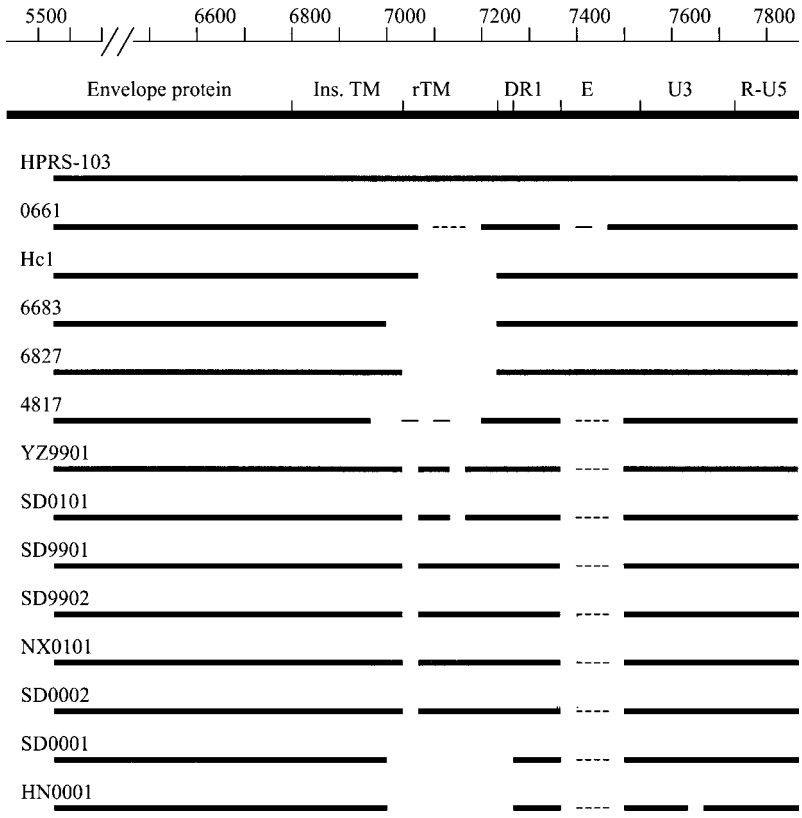

Fig. 2. Comparison of deletions at the 3′ end in the genomic proviral DNA of eight Chinese and five U.S. ALV-J strains with the prototype HPRS-103. The top two lines represent the base numbers and elements on the genomic proviral DNA of HPRS-103. The deletions are indicated by empty spaces between the thick black lines. The thin lines and dashed lines in the empty spaces indicate there are some short fragments that match HPRS-103.

However, the NS/S ratios were high enough to demonstrate selection pressures on some specific variable regions (Table 4), implying that these regions may be the most likely targets for immune selective pressure. There were six small domains with very high NS/S ratios. They were located in amino acids (aa) 103–158 (NS/S = 38/13), aa 141–158 (NS/S = 12/1), aa 180–202 (NS/S = 21/6), aa 185–199 (NS/S = 18/2), aa 233–244 (NS/S = 8/3), and aa 211–222 (NS/S = 16/3). Interestingly, strain NX0101 was unique and contained five nonsynonymous changes within the region of amino acids 141–151 and was also the last strain isolated in 2001 in China.

DISCUSSION

It is only a little more than 10 yr since ALV-J was first isolated and characterized in the United Kingdom (9). The virus has evolved rapidly in both its pathogenicity and antigenicity. The molecular basis for the virus evolution was studied in many strains isolated in the last 10 yr in Great Britain and the United States. The phylogenetic relationships of different strains, on the basis of the nucleotide sequence of the *env* gene, have been described (16,18). As the second largest broiler producer in the world, China did not report any ALV-J infection until 1999 (6). Even today, no measures are being taken to control the ALV-J spread. During the last 3 yr, we isolated eight ALV-J strains from four provinces. In order to further understand virus evolution, these Chinese strains were compared at the molecular level with strains from other parts of the world.

In a previous report, the percentages of identity of *env* proteins gp85 and gp37 from nine U.S. strains isolated in 1993–97 were analyzed and compared

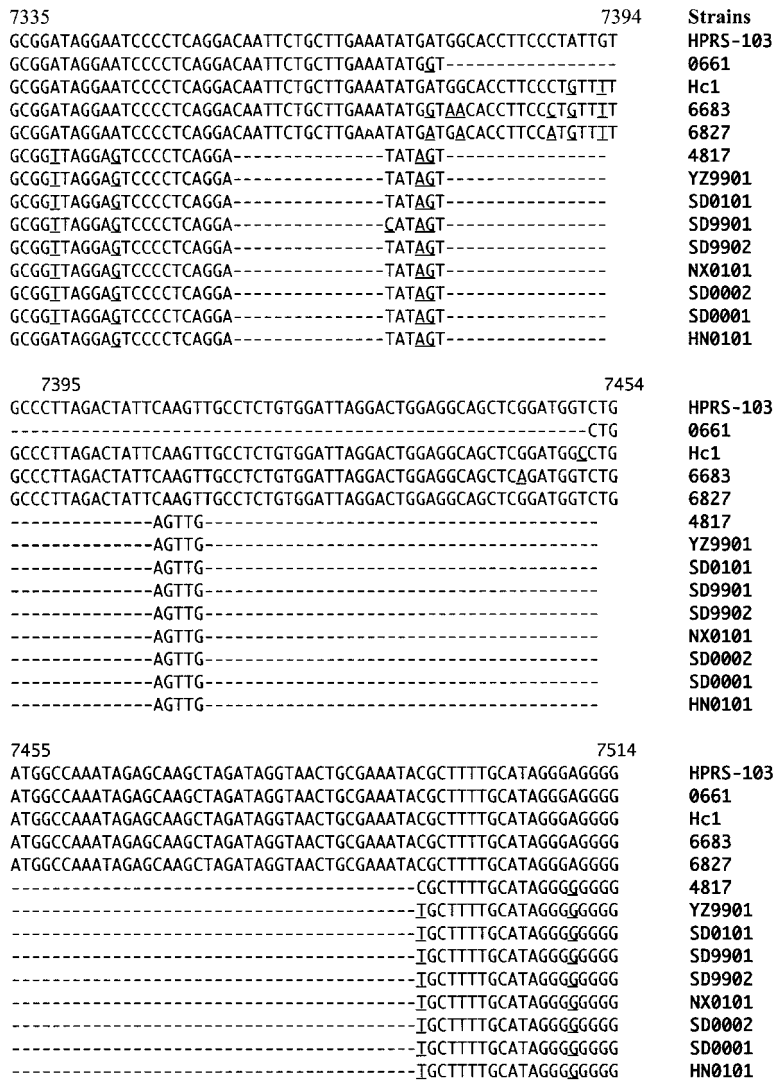

Fig. 3. DNA alignment of the sequences of the ALV-J noncoding regions. The DNA sequences are the region from DR1 to the end of the E element. The deleted sequences are represented by dashed lines. The bases not matching HPRS-103 are underlined.

with the ALV-J prototype U.K. strain HPRS-103 and endogenous sequence in line 0 cells. Over time, the U.S. strains have been slowly drifting away from their progenitor, found in line 0 CEFs (15,16). In this study, we compared Chinese strains of 1999–2001 and U.S. strains of 1993–97. In one sense, these new Chinese strains should be analogous to the latest emerging ALV-J in the U.S. because nearly all meat-type grandparent stocks in China are imported from the United States. Surprisingly, the eight Chinese strains were not more closely related to U.S. strains of 1997 but rather were more closely related to the earliest U.S. strain Hc1 and even the oldest prototype HPRS-103. In the case of the Chinese isolates, ALV-J does not appear to be drifting very rapidly away from HPRS-103 or Hc1. The suggestion has been made that the selection pressure from the immune response is driving the antigenic variation among ALV-J isolates (18) because most of the antigenic variants resisted neutralization with HPRS-103-specific serum. If correct, the Chinese viruses are evolving very slowly. If this trend continues, we may

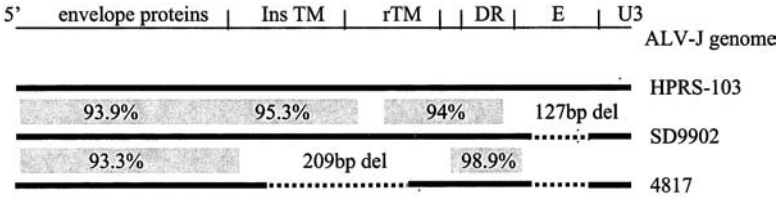

Fig. 4. Comparison of the 3′ end of the genomic proviral DNA in strains HPRS-103, 4817, and SD9902. The shadowed areas show the homology between two strains above and below the shadow (the percentage of identity is indicated in the shadows); the white areas demonstrate low homology between two strains.

see isolates that will be more closely related to the U.S. strains isolated in 1997.

Another common aspect of ALV-J evolution involves deletions in the 3′ end of genomic proviral DNA. All the Chinese and U.S. strains tested had one or two deletions in the rTM or E regions, relative to the prototype HPRS-103. All of these deletions overlapped to some extent between strains and were not quite identical. We found that all eight Chinese strains have the same deletion in E as the U.S. strain 4817, isolated in 1996. However, strain 4817 and the Chinese isolates differed in their deletions in the rTM region. Except for two or three bases, the deleted sequences in the E region of the eight Chinese strains and U.S. strain 4817 were almost identical (Fig. 3). Such similar deletions, which were isolated from different continents in different years, probably did not result from random mutation. Quite possibly, the U.S. strain 4817 and the eight Chinese isolates had a common progenitor. Their different patterns of rTM deletions suggest that 4817 and the Chinese isolates later diverged. It is also possible that there was some recombination between the strains. When the genomic proviral DNA of strains SD9902 and 4817 were compared with the prototype HPRS-103, there was 98.9% homology between 4817 and SD9902 at the right side of 3′-UTR. However, SD9902 was more closely related to HPRS-103 than to 4817 between the *env* and 3′-LTR (Fig. 4).

Table 4. Rates of amino acid substitutions in gp85 and its variable regions of 13 field strains compared with the ALV-J prototype HPRS-103.[A]

Region	No. amino acid changes		
	Nonsynonymous (NS)	Synonymous (S)	NS/S ratio
Total gp85	112	107	1.0
aa 103–158	38	13	2.9
aa 141–158	12	1	12.0
aa 180–202	21	6	3.5
aa 185–199	18	2	9.0
aa 233–244	8	3	2.3
aa 211–222	16	3	5.3

[A]The amino acid positions in the table correspond to the gp85 amino acid positions in the prototype strain HPRS-103.

The phylogenetic relationships among the eight Chinese isolates indicate that they are closely related to one another. Different regions of the genomic proviral DNA can evolve independently, thus, some Chinese strains, such as SD9901 and SD9902 isolated in 1999, showed high percentages of identity of 93.8%–94.2% in their gp85 amino acid sequences with prototype HPRS-103, but they were quite different from HPRS-103 at the 3′ end of the genomic proviral DNA (Fig. 4). Conversely, all eight Chinese strains had similar deletions in both rTM and the E elements and are similar to the U.S. strain 0661 isolated in 1996. Interestingly, the gp85 identity of strain 0661 with all eight Chinese strains was less than 89.1%. Consequently, it is necessary to compare both envelope protein amino acid percentage of identity and 3′ end of the genomic proviral DNA to more accurately establish the evolutionary relationships of ALV-J strains.

Unexpectedly, the oldest ALV-J, HPRS-103, was found to contain an "E" element. Previously, the E element was found only in Rous sarcoma viruses, not ALVs (13,17). However, the E element in all eight Chinese strains and the U.S. strain 4817 was almost completely deleted. Nevertheless, SD9902 could still induce acute myelocytomas in birds (5). Thus, the E element does not appear to be necessary for the acutely transforming ability of ALV-J. In addition, the rTM region of all the Chinese isolates had various portions deleted, suggesting that a "complete" rTM is not needed for viral replication or spreading of the virus in flocks.

Among the avian retroviruses, ALV-J appears to be the most variable, with frequent mutations

occurring in its envelope genes. As with other hypervariable RNA viruses, such as human immunodeficiency virus, ALV-J probably generates variable quasispecies in the infected individuals and populations. Although the mutations are somewhat random, the selection pressure undoubtedly influences the generation of the dominating quasispecies. Venugopal and coworkers (18) studied the selection pressures on the *env* gene and, in particular, the hr1, hr2, vr3 regions of the gp85. With the NS/S ratios of nucleotide mutations at the codon level (14), they concluded there were some selection pressures on the *env* gene of ALV-J. In the hr2 and vr3 regions of gp85, the NS/S ratios were as high as 7.0 and 4.50, respectively. Because Venugopal had already demonstrated that the gp85 amino acid changes were much more influenced by selection pressures than were sequences in gp37, we only analyzed selection pressures on gp85. Compared with the previous study by Venugopal et al. (18) with 12 U.K. field strains isolated in 1994–95, we analyzed 13 strains in this study, isolated from 1993 to 1997 in the United States and from 1999 to 2001 in China. We found 219 mutated sites in the gp85 of our 13 strains (Table 4). Although we found more mutation sites than the 130 sites found in the 12 U.K. field strains (18), the NS/S ratio was as low as 1.05, indicating no evidence of selection pressures on the total gp85. However, amino acids 103–158 of our 13 strains had many more mutated sites (51 sites) and a higher NS/S ratio of 2.92 (Table 4) than the 12 U.K. strains of 21 and 1.63, respectively. Additionally, the three most variable domains, with the highest NS/S ratios of 12, 9, and 5.3, were in amino acids 141–158, 185–199, and 211–222, respectively (Table 4). Our results clearly indicated more mutations and stronger selection pressures on specific regions of gp85 during the ALV-J evolution in the last several years.

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